September 4, 1948.

Dear Ed,

P

For the past few weeks, Mr. Zinder and I have been working on a selective method for isolating coli and Salmonella mutants which seems to be working very successfully in reconstruction experiments. Enclosed are "before-and-after" prints of EMB Lac plates showing the operation of the method on a mixture of K-12 and Y-53. A ratio of +:- of about 400:1 ended up as about 1:1, and we have had runs which were even more startling!

The method consists of inoculating the mixture of wild type and mutant (i.e. the irradiated suspensions) rather heavily (1:20) into minimal medium with 50-300 OU/ml penicillin, and incubating for 4 hours. Then dilute and plate to recover mutants. Layer plating may be unnecessary. The principle is that penicillin has a bactericidal action only on growing cells, or rather that there is a strong differential killing. Therefore, if washed cells are inoculated into synthetic medium, the wild types are killed about twice as fast as the mutants, and with a survival of 10^{-4} wild type there will be 10^{-2} mutants, a relative augmentation of 100:1, which has been the usual result, and should be satisfactory enough with available mutagens. With the method, we have only just begun to isolate new mutants, and have gotten quite a few in S. typhimurium, which seems to be somewhat more refractory even than coli. However, we still have to look into some further details to see whether improvement is possible. Unfortunately, the other antibiotics more active on coli supposedly have little bactericidal effect.

The principle should be applicable to Neurospora, if Ray can suggest some fungicides which have a comparable differential killing effect.

Work on the heterozygote is beginning to clear up some of the confusion. The heterozygous prototrophs can be maintained indefinitely as such on minimal agar. Segregants from the original W-465, when outcrossed continue to yield prototrophs heterozygous for Lac about 10% of the time. In these derived heterozygotes, there is some indication of heteroploidy, ie, that Mal is represented only once (either as - or +) although Lac is diplogenic as shown by its segregation. If W-465 is the zygote, it is probably tied up with a chromosomal aberration, but that will not diminish its usefulness for genetic analysis.

I hope you've been able to find another house by now, and that your lab will be set up pronto. We're all well. Best regards,

Yours sincerely